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Cutaneous and Respiratory Water Loss in Larks from Arid and Mesic Environments

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ABSTRACT

Birds from deserts generally have lower total evaporative water loss (TEWL), the sum of cutaneous (CWL) and respiratory water loss (RWL), than species from mesic areas. We investigated the role of CWL and RWL as a function of air temperature (T_a) in hoopoe larks (*Alaemon alaudipes*) and Dunn's larks (*Eremalauda dunni*) from the Arabian Desert and skylarks (*Alauda arvensis*) and woodlarks (*Lullula arborea*) from temperate mesic grasslands. The proportional contribution of CWL to TEWL in all larks at moderate T_a ranged from 50% to 70%. At high T_a (40°–45°C), larks enhanced CWL by only 45%–78% and relied on an increase in RWL by 676%–2,733% for evaporative cooling. Surface-specific CWL at 25°C was 29% lower in the arid-zone species than in the mesic larks. When acclimated to constant T_a , 15°C-acclimated hoopoe larks increased CWL by 22% compared with 35°C-acclimated birds, but the other species did not change CWL. This study is consistent with the hypothesis that larks from deserts have a reduced CWL at moderate and low T_a but provided no support for the hypothesis that at high T_a larks from arid regions rely more on CWL than larks from mesic environments. Interspecific differences in CWL cannot be attributed to acclimation to environmental temperature and are possibly the result of genetic differences due to natural selection or of phenotypically plastic responses to divergent environments during ontogeny.

Introduction

Total evaporative water loss (TEWL), the sum of cutaneous water loss (CWL) and respiratory water loss (RWL), is the main

avenue of water loss in birds and of major importance in maintaining heat balance, especially for birds living in hot and dry environments (Bartholomew 1972; Dawson 1982; Williams and Tieleman 2001). Birds from deserts generally have lower TEWL than species from mesic areas (Williams 1996), although the mechanisms responsible for this finding remain unresolved. Understanding these mechanisms might provide insights into the evolutionary processes that underlie the correlation between TEWL and environment. The variation in physiological traits such as TEWL and their adjustment to environmental conditions may be the result of genetic differences or phenotypic plasticity. Forms of plasticity include acclimatization or phenotypic flexibility of adult phenotypes in response to changing environments and developmental plasticity that can determine an individual's phenotype during ontogeny. Genetic variation in physiological traits may result from natural selection or genetic drift or may be a consequence of phylogenetic constraint (Gould and Lewontin 1979).

To minimize the confounding effect of phylogenetic factors, we compared TEWL and basal metabolic rate (BMR) within a closely related group of birds, all members of the lark family (Alaudidae), that are distributed over a gradient from mesic to arid habitats. We found a decline in TEWL and BMR with increasing aridity (Tieleman et al. 2002a, 2002b). To investigate the extent to which interspecific differences in TEWL and BMR result from acclimatization, we acclimated five species of larks to 15°C and 35°C (Tieleman et al. 2003). Hoopoe larks (*Alaemon alaudipes*) and Dunn's larks (*Eremalauda dunni*) live in arid deserts, spike-heeled larks (*Chersomanes albofasciata*) inhabit semiarid areas, and skylarks (*Alauda arvensis*) and woodlarks (*Lullula arborea*) dwell in mesic environments. When measured at 25°C, the 15°C-acclimated hoopoe larks increased their mass-specific TEWL by 23% compared with the 35°C-acclimated individuals, but the other species did not alter their TEWL in response to acclimation (Williams and Tieleman 2000; Tieleman et al. 2003). All species increased their BMR when acclimated to 15°C compared with 35°C. If metabolic rate is correlated with RWL, and therefore RWL has increased in the 15°C-acclimated birds, the fractional contributions of CWL and RWL to TEWL may have changed in these larks, even if TEWL is not different.

Several mechanisms have been proposed to explain the reduced TEWL in desert birds: hyperthermia (Calder and King 1974; Weathers 1981; Dawson 1984), countercurrent heat exchange in the nasal passages that lowers RWL (Schmidt-Nielsen et al. 1970), and adjustment of the lipid structure in

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the skin to reduce CWL (Menon et al. 1989, 1996). Previously, we explored the role of hyperthermia (Tieleman and Williams 1999) and of water recovery in the nasal turbinates in reducing TEWL in birds (Tieleman et al. 1999). Based on this work, we concluded that these factors could not account for the difference in TEWL between desert and nondesert forms.

Desert birds could reduce their TEWL by decreasing their CWL (Menon et al. 1989, 1996; Williams 1996). Although early investigators surmised that most evaporative cooling takes place in the respiratory passages (Rawles 1960; Bartholomew and Cade 1963; Mount 1979), later work showed that CWL is an important avenue of water loss in the thermoregulatory process, at least at air temperatures (T_a) below body temperature (Bernstein 1969; Smith 1969; Dawson 1982; Webster and Bernstein 1987; Webster and King 1987; Wolf and Walsberg 1996; Michaëli and Pinshow 2001). Collating data from the literature, we were unable to identify any significant differences in CWL at thermally neutral T_a between species from arid and mesic environments (Williams and Tieleman 2001). However, conclusions were tentative because data were few and were obtained in a variety of ways (Appleyard 1979; Marder and Ben-Asher 1983; Withers and Williams 1990).

Few studies have investigated CWL and RWL at high T_a when T_b must be regulated below lethal limits solely by evaporative water loss from the skin and from the respiratory passages (Marder and Ben-Asher 1983; Wolf and Walsberg 1996; Hoffman and Walsberg 1999). Some species, especially members of the Columbiformes, seem to rely primarily on CWL when T_a exceeds T_b , whereas other species seem to emphasize the use of RWL, facilitated by panting or gular flutter (Bouverot et al. 1974; Wolf and Walsberg 1996; Hoffman and Walsberg 1999; Tieleman et al. 1999; Williams and Tieleman 2001). Our understanding of CWL and RWL at high T_a and how water loss is partitioned remains rudimentary.

Our hypothesis is that natural selection should have equipped desert birds with a mechanism that impedes water loss through the skin, at least at moderate T_a , to save water. Under heat stress, when evaporative cooling is crucial for survival, evaporation through the skin should be elevated. Birds from mesic environments, however, which experience neither water scarcity nor high T_a , would not have been selected for reduced CWL at moderate T_a or for the capacity to enhance evaporation at high T_a . Rates of CWL in desert birds may be governed by two selective pressures: a long-term requirement of minimizing CWL, and as a result TEWL, and a short-term goal of maintaining T_b below lethal limits during episodes of extreme heat by means of increased RWL and CWL. CWL may be influenced by the structure and content of lipids in the epidermis (Menon et al. 1989, 1996), changes in which could be directed by natural selection in birds of arid environments.

In this study, we investigated CWL and RWL as a function of T_a in hoopoe larks and Dunn's larks from the Arabian Desert and skylarks and woodlarks from temperate grasslands in the

Netherlands. Data on the complete heat balance, including metabolism, TEWL, T_b , and the dry heat transfer coefficient per species and on the phylogenetic relationships between these four larks can be found in Tieleman et al. (2002b). We examined if CWL and RWL at moderate T_a were lower in the two arid-zone larks than in the two mesic birds and if, when birds were heat stressed, CWL increased more in the arid-zone larks than in those from the mesic areas. In addition, we explored the phenotypic flexibility of CWL and RWL of all four species in response to acclimation to 15°C and 35°C.

Material and Methods

We mistnetted hoopoe larks (*Alaemon alaudipipes*, mass 36.5 ± 3.7 g, $n = 14$) and Dunn's larks (*Eremalauta dunni*, 20.5 ± 1.8 g, $n = 16$) in Mahazat as-Sayd, a reserve in the Arabian Desert (22°15'N, 41°50'E), and housed them in outdoor aviaries at the National Wildlife Research Center near Taif, Saudi Arabia. Average yearly rainfall in Mahazat equals 90 mm, and maximum T_a in July averages 40.2°C with some daily maxima reaching 50°C (National Wildlife Research Center, unpublished data). Temperatures in Taif resemble those in Mahazat during the summer. In the Netherlands, we netted skylarks (*Alauda arvensis*, 31.5 ± 2.9 g, $n = 14$) and woodlarks (*Lullula arborea*, 25.5 ± 1.0 g, $n = 14$) in the province of Drenthe (52°52'N, 06°20'E) and kept them in outdoor aviaries at the Zoological Laboratory of the University of Groningen, 30 km from the capture site. In Drenthe, rainfall averages 750 mm yr⁻¹, and mean maximum T_a in July is 21.7°C (Koninkrijk Nederlands Meteorologisch Instituut). We used an open-flow system for indirect calorimetry to measure CWL, RWL, and metabolic rate (MR) of all birds during daytime after they had been in captivity for 3–6 wk during June, July, or August of 1999–2001. Larks were fed ad lib. a mixture of insects and seeds. In addition, larks in the Netherlands had access to water ad lib. Hoopoe larks and Dunn's larks do not drink, even when provided with water or after rains when temporary pools are available (B. I. Tieleman and J. B. Williams, personal observation).

Previous investigators have used a two-compartment system with the head and neck of the bird extending through a dental-dam barrier to quantify CWL and RWL separately (Bernstein 1971; Webster and King 1987; Wolf and Walsberg 1996; Hoffman and Walsberg 1999). We developed a system that reduces the contribution of the eyes, head, and neck to RWL while measuring RWL and CWL simultaneously (Fig. 1). We placed a bird fitted with a plastic mask in a metabolism chamber into which dry, CO₂-free outside air was drawn. The mask covered the entire bill and was held snug against the front of the bird's head with strips of soft leather. Thin pieces of thread attached to the mask and tied behind the head held the mask in place. Because the mask left the eyes and head of the bird exposed to the chamber, evaporation from eyes and head contributed

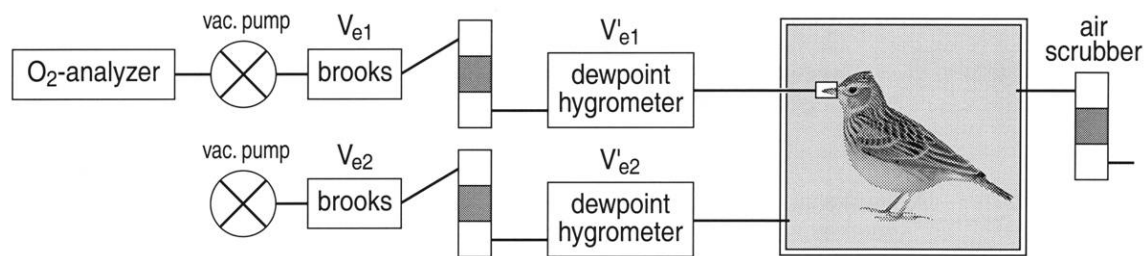


Figure 1. Laboratory setup to simultaneously measure cutaneous and respiratory water loss in birds

to CWL. Air flowed into the mask through spaces at the mask-forehead junction. Birds grew accustomed to wearing this setup in about 15 min and did not appear stressed when we removed them from the chamber. This method is an improvement over the chamber with a ventilatory and cutaneous partition. In the latter method, the bird is restrained with its head snug in a latex sheet, and “ventilatory” water loss includes evaporative water loss from respiratory passages, from the eyes, and from the head (Bernstein 1971; Webster and King 1987; Wolf and Walsberg 1996; Hoffman and Walsberg 1999).

In Saudi Arabia, steel metabolic chambers ($24 \times 20 \times 28$ cm) had an airtight Plexiglas lid and were water jacketed to control T_a by a Neslab circulating water bath (RTE-140) $\pm 0.2^\circ\text{C}$. In the Netherlands, larks were placed in steel metabolic chambers of identical dimensions, but T_a was controlled by a Heraeus Vötsch environmental chamber. Before measurements, birds had fasted 3 h. Birds were then placed on a wire-mesh platform over a layer of mineral oil that trapped excrement, thus excluding feces as a source of water in the measurements. Birds became accustomed to experimental conditions in about 15 min and generally remained inactive during the measurement period, as judged from the stable and flat traces of δO_2 (i.e., the differential output of the two-celled O_2 analyzer) and dew point. Any activity showed up on the graphs within 3 min because the response time of the O_2 analyzer and dew-point hygrometer to changes in respiratory values of occasional activity by the bird was within a few minutes. After air passed through a column of Drierite, we measured the dew point from which we calculated the water-vapor density ($\rho_{v\text{-in}}$, STP) of inlet air. Air, pulled through the mask, contained all respiratory gases and was routed through PTFE tubing, a General Eastern dew-point hygrometer (M4), columns of Drierite and Ascarite to remove H_2O and CO_2 , a Brooks mass-flow controller (model 5850E) calibrated with a bubble meter (Levy 1964), and a vacuum pump into an oxygen analyzer (Applied Electrochemistry S3A-II in Saudi Arabia and Servomex Xentra 4100 in the Netherlands). The dew-point hygrometers were factory calibrated with a primary standard traceable to the National Institute of Standards and Technology. Their response times were less than 3 min for dew points of -20°C and faster at higher dew points.

We calculated the flow rate through the dew-point hygrometer (V'_{e1}) by adjusting the value recorded at the mass-flow controller for H_2O and CO_2 added (Tieleman et al. 2002b), the latter estimated assuming a respiratory quotient of 0.71 (King and Farner 1961). In practice, these adjustments were less than 1%. To calculate RWL, we used the equation $\text{RWL} = (\rho_{v\text{-mask}} - \rho_{v\text{-chamber}})(V'_{e1})$, where $\rho_{v\text{-chamber}}$ is the water-vapor density (g m^{-3} , STP) of air going into the mask from the chamber, $\rho_{v\text{-mask}}$ is the water-vapor density (STP) coming out of the mask, and V'_{e1} is the corrected flow rate at the dew-point hygrometer. Oxygen consumption was calculated with equation (4a) of Withers (1977). We used $20.08 \text{ J mL}^{-1} \text{ O}_2$ to convert oxygen consumption to heat production (Schmidt-Nielsen 1997).

Air was also drawn from the chamber through a second port and passed along a second train identical to the first, except that air from the vacuum pump was vented to the room. Calculation of CWL was complicated by the fact that air that contained water vapor from the skin was exiting through two ports. We calculated air flow leaving the chamber by summing the flow rates from the mask (V'_{e1}) and from the chamber (V'_{e2}) and determined CWL with the equation $\text{CWL} = (\rho_{v\text{-chamber}} - \rho_{v\text{-in}})(V'_{e1} + V'_{e2})$. After a 2–3 h equilibration period, we recorded percentage O_2 and dew points of inlet, chamber and mask air, the temperature of the air in the dew-point hygrometers, and T_a in the chamber using a Campbell Scientific datalogger (model 21X or CR23X). When, during the third hour of measurements, the traces for O_2 consumption and dew points were stable for at least 10 min, we noted these times and used these data for calculations. To investigate the relationship between CWL and RWL and T_a , we measured these variables over a range from 15° to 45°C in hoopoe larks ($n = 7$ at each T_a) and Dunn’s larks ($n = 6$) and from 15° to 40°C in skylarks ($n = 7$) and woodlarks ($n = 6$). Relative humidities in the chamber were below 16% at all temperatures for all species and were unlikely to have impeded CWL (Lasiewski et al. 1966). Relative humidities of the air in the mask were for all species below 20% at T_a ’s up to 35°C and between 13% and 32% at 40°C , values low enough not to restrict RWL (Lasiewski et al. 1966). At 45°C , the relative humidity of the air in the mask of hoopoe larks and Dunn’s larks varied between

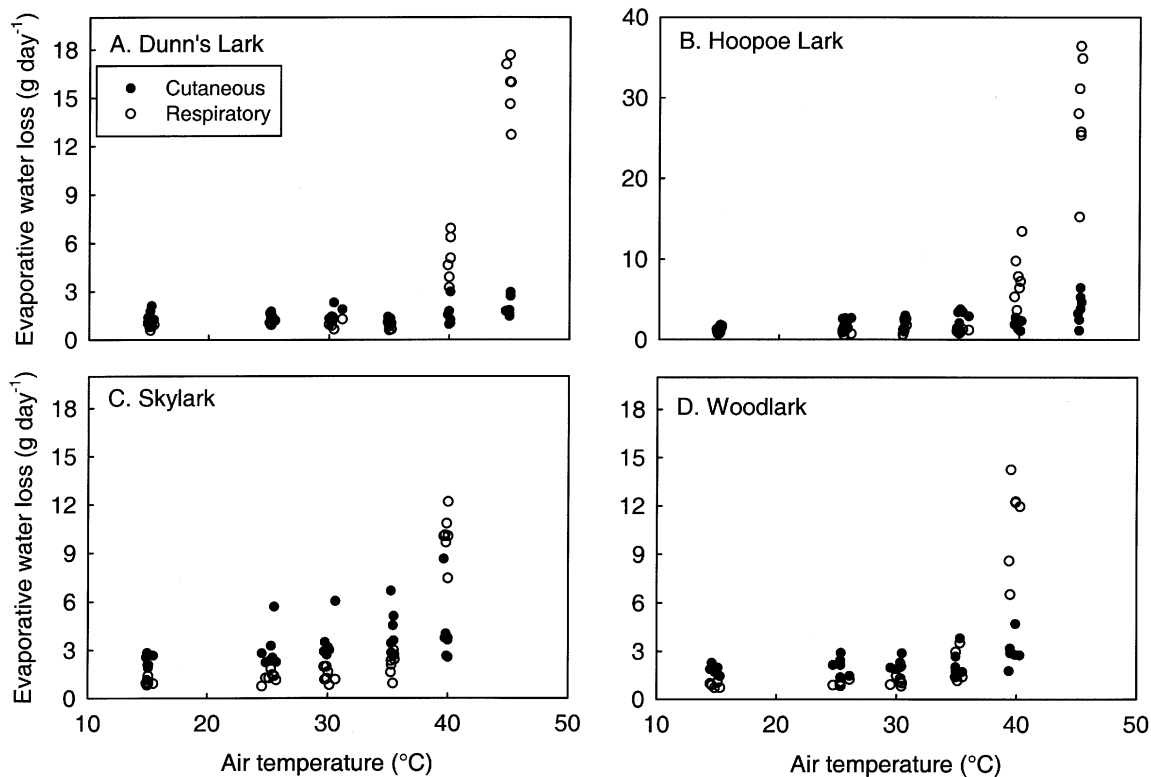


Figure 2. Cutaneous and respiratory water loss as a function of air temperature in Dunn's larks (A), hoopoe larks (B), skylarks (C), and woodlarks (D).

26% and 65%. Flow rates at this high T_a were a compromise between maintaining relative humidity sufficiently low not to impede RWL and δO_2 sufficiently high (above 0.09%) to obtain enough accuracy in measurements of O_2 consumption.

To ensure that all respiratory gases were captured by the mask, we set $V_{e1} > V_{e2}$. Depending on T_a and species, V_{e1} was set between 540 mL min⁻¹ and 1,475 mL min⁻¹ and V_{e2} between 360 mL min⁻¹ and 510 mL min⁻¹. In preliminary testing of our system, we placed a bird with a mask in the chamber and verified that the O_2 concentration of the air stream leaving the chamber always equaled the O_2 concentration of air entering the chamber, indicating that expired air of the bird was not mixing with the chamber air. Further, we confirmed that measurements of TEWL, given as the sum of RWL and CWL in this system, were not different from measurements of TEWL on the same individual birds when a conventional system was used (paired $t = 1.56$, $df = 24$, $P = 0.13$). Likewise, we confirmed that metabolic rates (MR) were indistinguishable between both systems (paired $t = 1.72$, $df = 24$, $P = 0.10$). Therefore, our system did not appear to be more stressful than the conventional metabolic chamber system.

To assess the flexibility of CWL and RWL, we measured TEWL and MR of skylarks and woodlarks at night and CWL,

RWL, and MR of hoopoe larks and Dunn's larks during the day at 25°C before we randomly assigned individuals to two groups with equal proportions of males and females and verified that the birds were similar in body mass in both assemblages. One group of each species was housed in a room with a constant T_a of 15° ± 2°C (12L : 12D), below the thermoneutral zone of all species involved and close to the average environmental T_a experienced by larks in the Netherlands during the breeding season. We placed the other group in rooms with a constant T_a of 35° ± 2°C (12L : 12D) to mimic environmental T_a of the Arabian Desert during spring. Birds were housed in groups of seven or eight individuals in cages of 1 × 1 × 2 m in the environmental rooms. Humidities were not controlled but were recorded on three occasions in Saudi Arabia with a Vaisala relative humidity probe and on two occasions in the Netherlands with the General Eastern dew-point hygrometer. The Vaisala relative humidity probe is generally not as accurate as the dew-point hygrometer, but we calibrated the probe over a NaCl solution and against the dew-point hygrometer and found it to be consistent with the latter. Absolute humidities were about 5 g m⁻³ and 7 g m⁻³ in the 15°C rooms and 12 g m⁻³ and 9 g m⁻³ in the 35°C rooms in Saudi Arabia and the

Netherlands, respectively. After 3 wk, we measured final values for CWL, RWL, and MR at 25°C for all birds during the day.

ANOVA and post hoc tests were carried out using general linear models procedures in SPSS 10.0. Averages are reported ± 1 SD. Experiments were performed under license of the University of Groningen (DEC 2425).

Results

All larks maintained low and relatively constant levels of CWL and RWL up to 35°C, but RWL increased rapidly at higher T_a (Fig. 2). Comparing values at 25°C and 40°C, CWL remained unchanged in Dunn's larks (1.51 ± 0.53 g d⁻¹; paired $t = 0.60$, $df = 5$, $P = 0.57$) and hoopoe larks (2.04 ± 0.55 g d⁻¹; paired $t = 0.27$, $df = 5$, $P = 0.80$); increased by 72% in skylarks, from 2.56 ± 0.41 g d⁻¹ to 4.39 ± 2.15 g d⁻¹ (paired $t = 2.01$, $df = 5$, $P = 0.10$); and by 45% in woodlarks, from 2.07 ± 0.58 g d⁻¹ to 3.01 ± 0.96 g d⁻¹ (paired $t = 3.09$, $df = 5$, $P = 0.03$). In contrast, RWL increased rapidly at high T_a in all species. Comparing RWL at 25°C and 40°C, values increased by 332% in Dunn's larks, from 1.16 ± 0.20 g d⁻¹ to 5.01 ± 1.41 g d⁻¹ (paired $t = 6.02$, $df = 5$, $P = 0.002$); by 665% in hoopoe larks, from 1.00 ± 0.35 g d⁻¹ to 7.63 ± 3.49 g d⁻¹ (paired $t = 4.59$, $df = 5$, $P = 0.006$); by 676% in skylarks, from 1.28 ± 0.36 g d⁻¹ to 9.92 ± 1.51 g d⁻¹ (paired $t = 12.58$, $df = 5$, $P < 0.0001$); and by 993% in woodlarks, from 1.00 ± 0.15 g d⁻¹ to 10.97 ± 2.84 g d⁻¹ (paired $t = 8.52$, $df = 5$, $P < 0.0001$). Adjusting the significance level of the t -tests with a sequential Bonferroni's correction did not alter the results (Rice 1989). Dunn's larks and hoopoe larks did not increase CWL and RWL at 40°C by as much as skylarks and

woodlarks. However, at 45°C, Dunn's larks and hoopoe larks had increased RWL by 1,248% and 2,733% compared with values at 25°C, whereas CWL only increased by 75% and 78%, respectively.

When T_a increased from 15° to 35°C, CWL varied from 55% to 70% of TEWL in all four species (Fig. 3). When T_a exceeded 35°C, all species increased RWL considerably, and the contribution of CWL to TEWL decreased to around 25% at 40°C. Hoopoe larks and Dunn's larks increased RWL even further at 45°C, where CWL constituted only 12% of TEWL. Therefore, we conclude that in all four lark species CWL is a significant proportion of TEWL at moderate and low T_a but that RWL is the primary avenue for evaporative heat loss at high T_a .

To normalize CWL and RWL, we calculated surface-specific CWL with Meeh's equation for skin surface area (Walsberg and King 1978) and mass-specific RWL by dividing RWL by body mass (Fig. 4). At 25°C, surface-specific CWL and mass-specific RWL differed significantly between species (CWL, species $F_{3,22} = 3.44$, $P = 0.034$; RWL, species $F_{3,22} = 7.69$, $P = 0.001$). Post hoc analysis showed a lower surface-specific CWL in hoopoe larks than in skylarks and no difference between Dunn's larks and hoopoe larks or between skylarks and woodlarks (Fig. 4A) and a lower mass-specific RWL in hoopoe larks compared with Dunn's larks (Fig. 4C). To provide an estimate of the magnitude of differences between arid and mesic larks, we combined woodlarks and skylarks as mesic birds and Dunn's larks and hoopoe larks as arid birds. Surface-specific CWL was 26.5 ± 9.59 mg cm⁻² d⁻¹ in the former and 18.8 ± 3.25 mg cm⁻² d⁻¹ in the latter, a reduction of 29% in the desert species. Mass-specific RWL at 25°C averaged 39.0 ± 9.47 mg d⁻¹ g⁻¹ in the mesic larks and 40.6 ± 11.64 mg d⁻¹ g⁻¹ in the desert birds, almost identical values. At 40°C, the highest T_a with data for all larks, species also differed significantly in surface-specific CWL and mass-specific RWL (CWL, species $F_{3,22} = 4.72$, $P = 0.011$; RWL, species $F_{3,22} = 9.86$, $P < 0.0001$). Skylarks had a higher surface-specific CWL than Dunn's larks and hoopoe larks but did not differ from woodlarks (Fig. 4B). Woodlarks had a higher mass-specific RWL than Dunn's larks and hoopoe larks but did not differ from skylarks, whereas hoopoe larks had a lower RWL than both mesic species but were not different from Dunn's larks (Fig. 4D). When we compared the average surface-specific CWL and the mass-specific RWL of the two arid species combined (CWL, 18.8 ± 8.54 mg cm⁻² d⁻¹; RWL, 215.7 ± 72.23 mg d⁻¹ g⁻¹) with the values of the two mesic species combined (CWL, 37.5 ± 16.51 mg cm⁻² d⁻¹; RWL, 356.2 ± 92.53 mg d⁻¹ g⁻¹) to obtain an idea of the magnitude of differences between larks from both environments, we found reductions in the arid birds of 50% and 39%, respectively.

Because skylarks and woodlarks have higher rates of metabolism than Dunn's larks and hoopoe larks (Tieleman et al. 2002b), we also compared evaporative water loss per unit energy expenditure among species (Fig. 5). CWL normalized for metabolism (milligrams water per kilojoule) increased little with

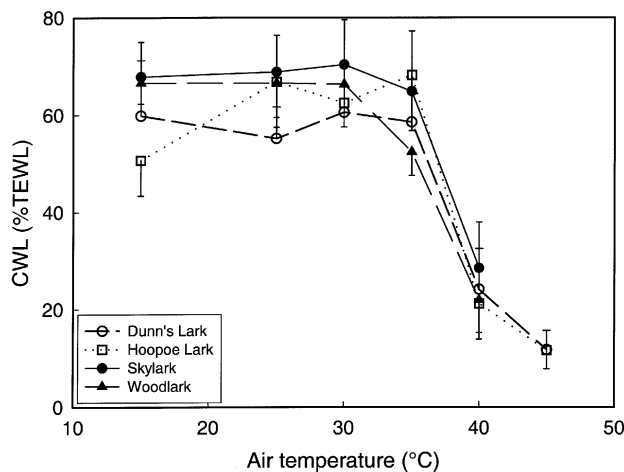


Figure 3. Proportional contribution of cutaneous water loss (CWL) to total evaporative water loss (TEWL) as a function of air temperature in Dunn's larks, hoopoe larks, skylarks, and woodlarks. Error bars indicate 1 SD.

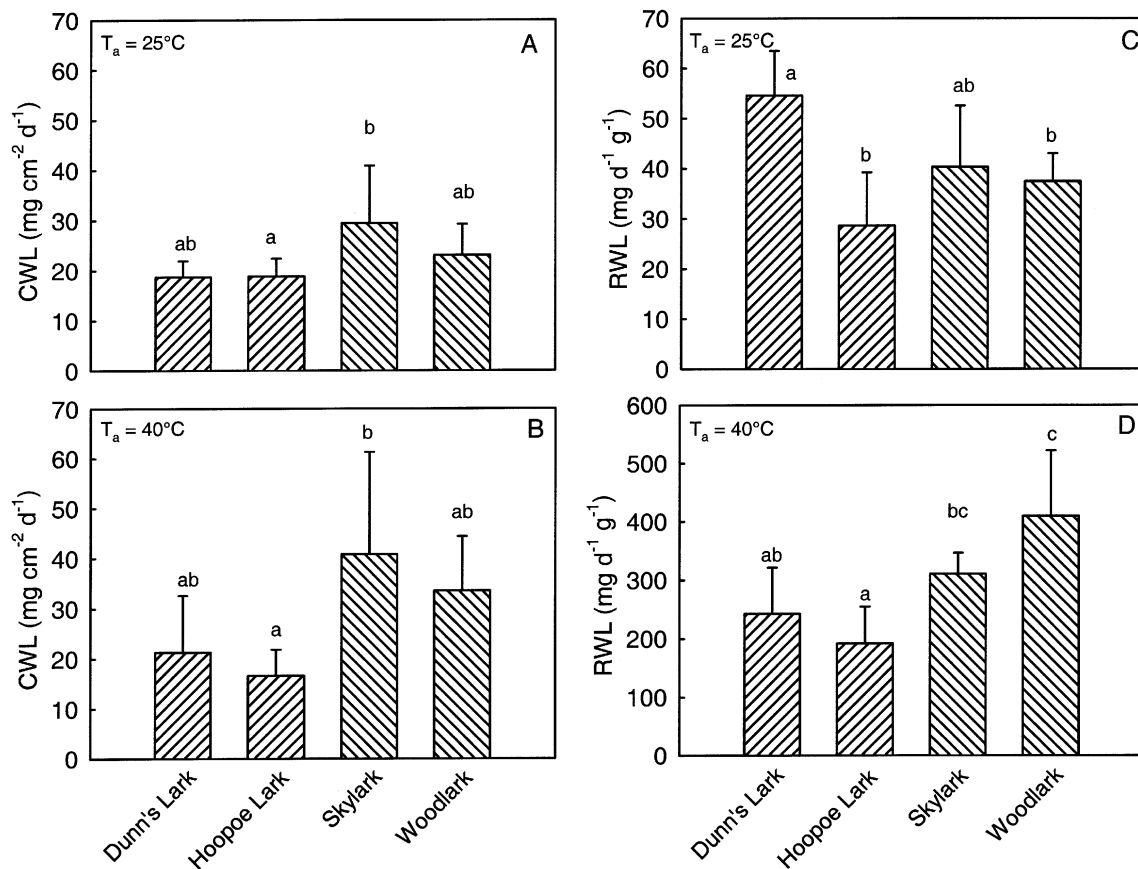


Figure 4. Surface-specific cutaneous water loss (CWL; mean \pm 1 SD) at 25°C (A) and 40°C (B) and mass-specific respiratory water loss (RWL; mean \pm 1 SD) at 25°C (C) and 40°C (D) of Dunn's larks, hoopoe larks, skylarks, and woodlarks. Identical letters indicate that bars were not significantly different from each other when analyzed with a Tukey test following a univariate ANOVA (critical $P = 0.05$).

increasing T_a and was 61% higher at 40°C than at 25°C for all species combined (Fig. 5A). It did not differ among species at either 25°C ($F_{3,22} = 1.08$, $P = 0.38$) or 40°C ($F_{3,22} = 0.87$, $P = 0.47$). In all four species, RWL normalized for metabolism (milligrams water per kilojoule) increased little between 15°C and 35°C but rapidly when T_a increased to 40° and 45°C (Fig. 5B). At 25°C, normalized RWL differed significantly between species ($F_{3,22} = 6.12$, $P = 0.003$), and post hoc analysis showed that normalized RWL of Dunn's larks was higher than that of hoopoe larks (Tukey $P = 0.037$), skylarks (Tukey $P = 0.014$), and woodlarks (Tukey $P = 0.003$) but that the other three species were indistinguishable from each other (Tukey all $P > 0.63$). At 40°C, differences in normalized RWL between species were significant ($F_{3,22} = 4.35$, $P = 0.015$), and post hoc analysis showed that RWL of the woodlark was higher than that of the skylark (Tukey $P = 0.011$) but that the other species pairs did not differ (Tukey all $P > 0.07$).

CWL, measured at 25°C, of hoopoe larks acclimated at 15°C was significantly higher by 22% than that of conspecifics ac-

climated at 35°C, but CWL of Dunn's larks, skylarks, and woodlarks did not differ between treatments (hoopoe larks, $F_{1,11} = 4.92$, $P = 0.049$; Dunn's larks, $F_{1,13} = 0.05$, $P = 0.82$; skylarks, $F_{1,11} = 2.94$, $P = 0.11$; woodlarks, $F_{1,11} = 1.67$, $P = 0.22$; Fig. 6A). RWL, measured at 25°C, did not differ between larks acclimated to 15° and 35°C in any of the four species (hoopoe larks, $F_{1,11} = 3.61$, $P = 0.08$; Dunn's larks, $F_{1,13} = 0.004$, $P = 0.95$; skylarks, $F_{1,11} = 2.43$, $P = 0.15$; woodlarks, $F_{1,11} = 0.02$, $P = 0.88$; Fig. 6B). Although metabolic rates of skylarks and Dunn's larks acclimated to 15°C were significantly higher than those of individuals acclimated to 35°C (Table 1), we did not find differences in RWL. Neither hoopoe larks nor woodlarks acclimated to 15° and 35°C differed in metabolic rate (Table 1).

Discussion

CWL and RWL as a Function of T_a

When exposed to moderate and low T_a , hoopoe larks, Dunn's larks, skylarks, and woodlarks maintained relatively low and

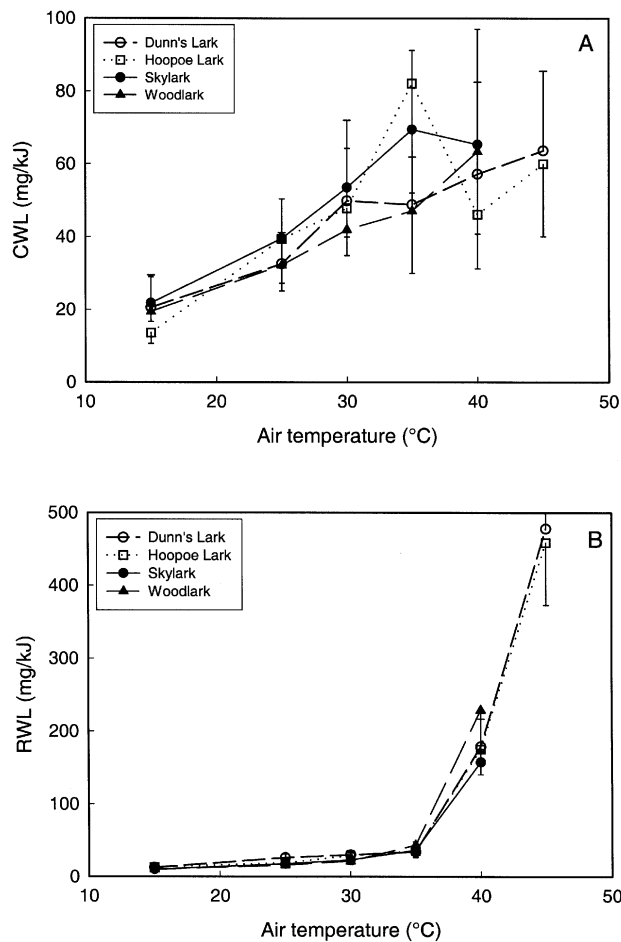


Figure 5. Energy-specific cutaneous (A) and respiratory (B) water loss as a function of air temperature in Dunn's larks, hoopoe larks, skylarks, and woodlarks. Error bars indicate 1 SD.

constant levels of CWL and RWL, but when T_a exceeded 35°C, only RWL rapidly increased (Fig. 2). The contribution of CWL to TEWL at T_a ranging from 15° to 35°C was 50%–70% in all four larks, values within the range reported for the few other species measured, including the similar-sized passerines budgerigah (*Melopsittacus undulatus*), sociable weaver (*Ploceus cucullatus*), and zebra finch (*Taeniopygia guttata*; Bernstein 1971; Calder and King 1974; Dawson 1982; Marder and Ben-Asher 1983; Webster et al. 1985). When T_a exceeded 35°C, all larks increased reliance on RWL for thermoregulation. The contribution of CWL to TEWL decreased to around 25% at 40°C and to 12% at 45°C, the latter for Dunn's larks and hoopoe larks.

At 25°C, CWL of hoopoe larks (2.16 g d⁻¹) and Dunn's larks (1.43 g d⁻¹) were 17% and 15% below allometric predictions, respectively, whereas CWL of skylarks (2.56 g d⁻¹) and woodlarks (2.07 g d⁻¹) were 11% and 3% above allometric predic-

tions (Williams and Tieleman 2001). Surface-specific CWL at 25°C was 29% lower in the two arid-zone larks than in the two species from mesic habitats, but mass-specific RWL did not differ. These results are consistent with the hypothesis that, at moderate T_a , CWL is reduced in birds from arid environments, a reduction that accounts for most of the difference in TEWL between desert and nondesert birds. Mass-adjusted TEWL at 25°C was 27% lower in Dunn's larks and hoopoe larks compared with skylarks and woodlarks (Tieleman et al. 2002b).

When heat stressed, woodlarks and skylarks at 40°C increased their CWL by 45% and 72% compared with 25°C, whereas hoopoe larks and Dunn's larks at 45°C increased their CWL by 78% and 75%, respectively. This pattern differs from that of pigeons and doves, chukar (*Alectoris chukar*), spotted sandgrouse (*Pterocles senegallus*), and rhea (*Rhea americana*), where the increase in CWL at 45°C is 230%–1104% (Marder and Ben-Asher 1983; Hoffman and Walsberg 1999; Williams and

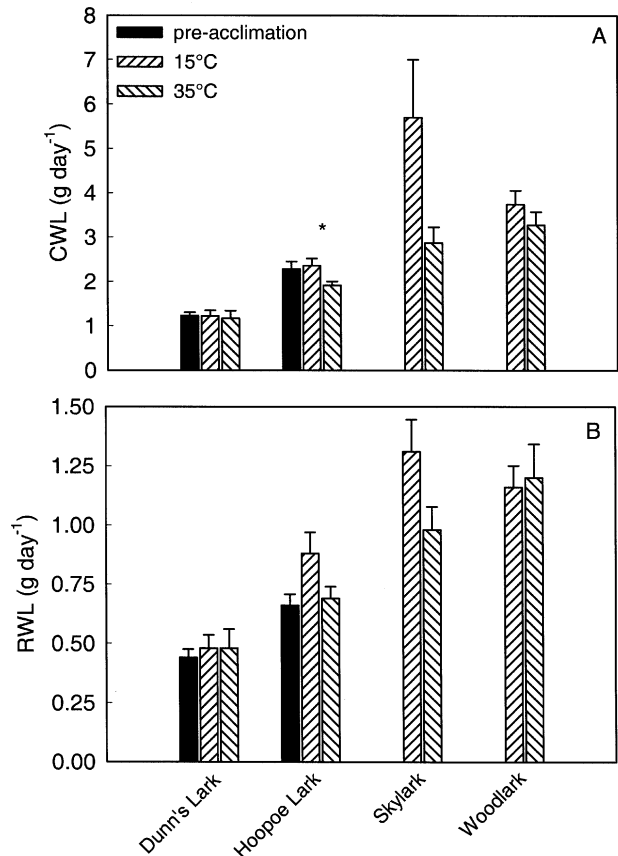


Figure 6. Cutaneous (CWL; A) and respiratory (RWL; B) water loss (mean \pm 1 SEM) of Dunn's larks, hoopoe larks, skylarks, and woodlarks when acclimated to the outside environment (preacclimation) and after a 3-wk acclimation period in constant temperature rooms of 15° or 35°C. Asterisk indicates significant difference in CWL between 15°C- and 35°C-acclimated hoopoe larks.

Table 1: Metabolic rate (MR; kJ d⁻¹) at 25°C of Dunn's larks, hoopoe larks, skylarks, and woodlarks acclimated to 15° or 35°C

Species	15°C			35°C			F	P
	MR (kJ d ⁻¹)	SD	n	MR (kJ d ⁻¹)	SD	n		
Dunn's lark	49.7	3.17	8	44.8	3.38	8	10.14 _{1, 13}	.007
Hoopoe lark	67.7	10.56	7	62.4	5.78	7	1.43 _{1, 11}	.26
Skylark	113.8	15.25	7	90.3	11.94	7	10.50 _{1, 11}	.008
Woodlark	91.8	5.38	7	88.1	6.20	7	.59 _{1, 11}	.46

Tieleman 2001). All four species of larks relied less on CWL at high T_a than did the verdin (*Auriparus flaviceps*), the only other passerine for which CWL has been measured at high T_a , which increased its CWL by 122% (Wolf and Walsberg 1996). We found no support for the hypothesis that larks from deserts increase their CWL at high T_a more than nondesert larks. Instead, when heat stressed, all larks relied on RWL for evaporative cooling. CWL has been assumed to be more efficient than RWL at high T_a because CWL is a passive diffusion process that does not require muscle activity. Increased RWL by panting and gular flutter would require muscle activity and therefore result in increased heat production (Arad et al. 1987). However, Hoffman and Walsberg (1999) suggest that ultimately both routes of evaporative heat loss are passive diffusional processes, and although CWL may not require muscular activity, it needs delivery of water to the stratum corneum, a process that could be energetically costly. The combination of high CWL and high MR of mesic larks and that of low CWL and low MR of arid larks resulted in indistinguishable rates of energy-specific CWL. However, assessing the energetic costs of CWL will require an experimental approach.

Phenotypic Flexibility of CWL and RWL

The reduced CWL of hoopoe larks and Dunn's larks compared with skylarks and woodlarks could result from genetic differences brought about by natural selection or drift or by phenotypic responses to different environments during either ontogeny or adult life. Only the hoopoe lark increased its CWL by 22% when acclimated to 15°C compared with 35°C, but the difference in CWL was too small to account for the interspecific differences in CWL, suggesting that acclimatization to T_a plays a minor role in determining CWL. Possible effects of the humidity of the environment, of water availability in food items or as drinking water, or of conditions during development remain to be studied in birds. In Merriam's kangaroo rats (*Dipodomys merriami*), adults from mesic and arid sites differed in CWL, but the arid-site individuals increased CWL to the same level as the mesic-site animals when acclimated to constant humidity and T_a in the laboratory (Tracy and Walsberg 2000). Developmental plasticity and acclimation can account completely for the intraspecific differences in TEWL in this

species (Tracy and Walsberg 2001). Compared with adults, nestling zebra finches (*Taeniopygia guttata*) have a lower CWL and a higher lipid content in the epidermis, a structure that is thought to form the basis of the integumentary permeability barrier for water (Menon et al. 1987). However, evidence that CWL of adults may depend on developmental conditions is not available.

Potential Mechanisms

Mechanisms that might explain the difference in CWL between larks from arid and mesic habitats and that could account for the flexibility of CWL in the hoopoe lark might include alterations of the permeability of the skin to water vapor or of the diffusion path length. CWL is a function of the water-vapor gradient between skin and air and the total resistance to water-vapor diffusion across skin, feathers, and boundary layer (Appleyard 1979; Webster and King 1987; Wolf and Walsberg 1996). Resistance to vapor diffusion across the skin accounts for 75%–90% of the total, at least at moderate T_a (Tracy 1982; Marder and Ben-Asher 1983; Webster et al. 1985). For resistance across the skin to change, birds must vary the diffusion path length or alter the permeability of the skin to water vapor. The skin of birds is composed of an epidermis and a well-vascularized dermal layer (Lucas and Stettenheim 1972). During heat stress, birds can reduce the diffusion path length by vasodilation of the dermal capillary bed, effectively increasing CWL (Peltonen et al. 1998). Rock doves (*Columba livia*) under heat stress not only dilate capillaries but also increase the permeability of the skin to water vapor (Smith 1969; Arieli et al. 1995; Peltonen et al. 1998). In response to dehydration, changes in epidermal lipid conformation within the stratum corneum may reduce the permeability of the skin to water vapor, although data are few (Menon et al. 1987, 1989, 1996).

Evolutionary Perspective

Evaporative heat loss at high T_a follows contrasting pathways in birds from different taxonomic groups. Heat-stressed Columbiformes rely primarily on CWL (Marder and Ben-Asher 1983; Arad et al. 1987), whereas Passeriformes rely largely on RWL to dissipate excess heat (Wolf and Walsberg 1996; this

study). Although species from both taxa occur in deserts and are able to withstand high T_a , pigeons and doves rely on drinking water daily (Arad et al. 1987), but larks do not. When drinking water is denied, dehydrated rock pigeons diminish CWL, become hyperthermic, and develop signs of thermal distress (Arad et al. 1987). However, even after rainfall, we have never observed hoopoe larks and Dunn's larks drinking in the Arabian Desert, while many other birds do. We suggest that taxon-specific responses of CWL to high T_a might result from divergent selection for heat-tolerance mechanisms depending on whether species have access to drinking water or are selected for a frugal water economy. The generally low TEWL of larks (Tieleman et al. 2002a) may result from adjustments in the skin that do not allow a rapid increase in CWL under acute heat stress. Instead, when exposed to T_a exceeding T_b , larks rely on increasing RWL for thermoregulation.

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